View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by Infoscience - École polytechnique fédérale de Lausanne

Research Article

Journal of Molecular Recognition

Received: 22 September 2009, Revised: 8 January 2010, Accepted: 1 February 2010, Published online in Wiley Online Library: 7 April 2010

(wileyonlinelibrary.com) DOI:10.1002/jmr.1030

Force spectroscopy of barnase–barstar single molecule interaction†

S. K. Sekatskii[a]*, M. Favre[a], G. Dietler[a], A. G. Mikhailov[b], D. V. Klinov[b], S. V. Lukash[b] and S. M. Deyev[b]

Results of the single molecule force spectroscopy study of specific interactions between ribonuclease barnase and its inhibitor barstar are presented. Experimental data obtained for the force loading rate ranging 2–70 nN/s are well approximated by a single straight line, from which the dissociation barrier of the width of 0.12 nm and height of $0.75–0.85\times10^{-19}$ J can be inferred. The measured value of specific interaction does not depend on the NaCl concentration. This apparently contradicts the well-known dependence of the binding energy of this pair on the salt concentration, but such a "contradiction" is explained by the insensitivity of the force spectroscopy data to the relatively long-range electrostatic interaction. The latter essentially contributes to the value of barnase–barstar binding energy revealed by biochemical measurements, and it is exactly this electrostatic interaction which is influenced by the salt concentration. Copyright © 2010 John Wiley & Sons, Ltd.

Keywords: barnase; barstar; ligand-receptor interactions; force spectroscopy

INTRODUCTION

Nowadays, the Atomic Force Microscopy (AFM)-based single molecule force spectroscopy became a versatile research tool enabling to obtain, among others, important and often unique in comparison with other methods data concerning the energy landscape of ligand–receptor pairs. The most efficiently interacting avidin–biotin and streptavidin–biotin pairs naturally appeared as the first objects having been investigated by the single molecule force spectroscopy (Lee *et al*., 1994; Florin *et al*., 1994), and numerous other pairs have been studied later. Analysis of the literature (see, among others, recent reviews Zlatanova *et al*., 2000; Schwesinger *et al*., 2000; Weisel *et al*., 2003; Lee *et al*., 2007) reveals that the corresponding data is now available for many tens, if not hundreds, ligand–receptor pairs.

By these reasons the lack of the force spectroscopy data concerning the ribonuclease barnase (110 amino acids) and its inhibitor barstar (89 amino acids; see Hartley, 2001 for review) seems somewhat surprising. This protein pair is not only "the second most effectively interacting one" just after avidin/streptavidin–biotin (dissociation constant for the former is around 10^{-14} M (Mariani *et al*., 1992; Schreiber and Fersht, 1993; Hartley, 2001) while it is $\cong 10^{-15}$ M for the latter (Green, 1990), but it is much exploited in practice and is nowadays considered as a very prospective "constructor" to create multivalent complexes used as innovative protein therapeutics (Deyev *et al*., 2003). Both these proteins can be efficiently expressed, are stable and highly resistive to the harsh environmental conditions, and they are capable to reversible restoration of the structure and function after denaturation when an action of denaturating agents is removed. Their 3D structure is well known, and their association/dissociation reactions were much studied and modeled. It is also worthwhile to mention that barnase has been long considered as a kind of model system when the protein folding/defolding problem is concerned (Fersht, 1993).

All aforementioned circumstances make single molecule force spectroscopy study of barnase–barstar pair quite timely. Evidently, corresponding data is important for the better understanding of this system and hence for its biotechnological and medical applications. Another motivation for this study consists in the following. Experimental (Schreiber and Fersht, 1993; Schreiber and Fersht, 1995, 1996) and theoretical (Chong *et al*., 1998; Lee and Tidor, 2001a, 2001b; Schneierman and Honig, 2002; Dong *et al*., 2003; Wang *et al*., 2004; Spaar *et al*., 2006; Tang *et al*., 2006; Ababobu *et al*., 2007) data attest an important contribution of an electrostatic interaction to the barnase–barstar energy landscape. However, despite a lot of efforts spent for the corresponding modeling and calculations, even the sign of the effect still cannot be unambiguously predicted. Biochemical experiments clearly show a favorable contribution of an electrostatic interaction: the binding free energy in barnase–barstar complex reduces on the value of 3.1 kcal/mol for 500 mM NaCl concentration as compared with the pure water case (Schreiber and Fersht, 1993). At the same time, the

* *Correspondence to: S. K. Sekatskii, Laboratoire de Physique de la Matière Vivante, IPSB, BSP, Ecole Polytechnique Fédérale de Lausanne (EPFL), CH-1015 Lausanne, Switzerland.*
E-mail: serguei.sekatski@epfl.ch

a *S. K. Sekatskii, M. Favre, G. Dietler*
Laboratoire de Physique de la Matière Vivante, IPMC, BSP, Ecole Polytechnique Fédérale de Lausanne (EPFL), CH-1015 Lausanne, Switzerland

b *A. G. Mikhailov, D. V. Klinov, S. V. Lukash, S. M. Deyev*
M. M. Shemyakin and Yu. V. Ovchinnikov Institute of Bioorganic Chemistry Russian Academy of Sciences, 119991 Moscow, Russia

† *This article is published in Journal of Molecular Recognition as a special issue on Affinity 2009, edited by Gideon Fleminger, Tel-Aviv University, Tel-Aviv, Israel and George Ehrlich, Hoffmann-La Roche, Nutley, NJ, USA.*

Copyright © 2010 John Wiley & Sons, Ltd.

theoretical predictions concerning electrostatic interactions in the barnase–barstar pair are inconsistent: while a number of papers predict favorable electrostatic contributions to binding affinity (Lee and Tidor, 2001a; Dong *et al.*, 2003; Wang *et al.*, 2004; Spaar *et al.*, 2006), unfovarable (Lee and Tidor, 2001b) or net zero contributions (Schneierman and Honig, 2002) were also calculated. Furthermore, an analysis of the published computational results reveals their strong dependence on the details of the model exploited hence new researches in the field are needed.

We believe that the methods of single molecule force spectroscopy are quite appropriate just for the case: electrostatic interaction is characterized by a relatively long spatial scale very different from that of the short range "contact" hydrogen bonds-mediated interprotein interactions. Force spectroscopy, which is known to be sensitive to the width of the energy barrier, looks like a natural tool to distinguish between them. Experimental results obtained confirm our anticipations and this, we believe, opens new perspectives for a systematic use of the force spectroscopy methods to clarify the role of different contributions to the total complex energy.

MATERIALS AND METHODS

Barnase and barstar

Barnase and barstar were prepared as follows. Barnase was produced in TG-1 *E. coli* cells freshly transformed by the pPBn plasmid (Schulga *et al.*, 1998). Starter culture was grown overnight in YTPSG medium (1% yeast extract, 1% tryptone, 100 mM NaCl, 45 mM K_2HPO_4, 5 mM KH_2PO_4, 0.5% glucose, pH 7.4), 100 mg/L ampicillin at 28°C. The same medium (1 L) was inoculated with 5 ml of the overnight culture. The bacteria were grown at 28°C until the cell culture reaches $OD_{550} = 0.05$ and then during additional 12–15 h at 37°C. All the following operations were performed at 4°C. Glacial acetic acid was added to the bacterial culture at 50 ml/L. The suspension was stirred for 30 min. The cells were removed by centrifugation at 7000*g* for 10 min. Phosphocellulose P11 in 20 mM Na-acetate buffer, pH 4.5 was added to the supernatant and the suspension was stirred for 3–4 h. Phosphocellulose was transferred into a column washed with the same buffer, and barnase was eluted with a linear gradient of 20–800 mM NH_4-acetate buffer, pH8.0. Final purification of the barnase was performed on the column HiTrap 1 ml SP FF, equilibrated with 20 mM Na-acetate buffer, pH 5.5. Barnase was eluted with a linear salt gradient of 0–400 mM NaCl in 20 mM Na-acetate buffer, pH 5.5.

Barstar (C40,82A) was produced in HB101 *E. coli* cells freshly transformed the pMT643 plasmid (Jucovic and Hartley, 1996) and isolated according to modified procedure (Schreiber and Fersht, 1993). Starter culture was grown overnight in YTPSG medium (1% yeast extract, 1% tryptone, 100 mM NaCl, 45 mM K_2HPO_4, 5 mM KH_2PO_4, 0.5% glucose, pH 7.4), 100 mg/L ampicillin at 37°C. The overnight culture (5 ml) was added to 1 L YTPSM medium with the antibiotic and after 12 h cultivation at 37°C the bacterial cells were pelleted by centrifugation. All the following operations were performed at 4°C. Resuspended cell pellet in 30 ml ice-cold 50 mM Tris-HCl buffer, 100 mM NaCl, 10 mM EDTA, 10 mM DTT, pH8.0 was sonicated for 3–4 min on ice. The pellet was removed by centrifugation at 30 000*g* for 30 min. The supernatant was saturated with ammonium sulfate to 40% and centrifugated (30 000*g*, 30 min). The second supernatant was saturated with ammonium sulfate to 70–75% and again centrifuged for 30 min. The pellet was dissolved in 3–5 ml of 30 mM Tris-HCl buffer, 50 mM NaCl, 2 mM EDTA, 2 mM DTT, pH8.0. Proteins were fractionated by gel-filtration on Sephadex G100 SuperFine (16/100) column. Fractions containing barstar were collected and final purification was done on column HiTrap1ml Q Seph FF. Barstar was eluted with a linear salt gradient of 50–500 mM NaCl in Tris-HCl buffer, 2 mM EDTA, 2 mM DTT, pH8.0.

Proteins purity was verified by SDS-PAGE analysis.

AFM instrumentation

The same experimental setup and the procedure of tip and sample functionalization, which have been used for the single molecule force spectroscopy earlier and described in details in our papers (Chtcheglova *et al.*, 2004; Favre *et al.*, 2007), were exploited for this study which enables us to give here only a rather brief account. The data were obtained in Lausanne using the AFM Nanoscope IV "Picoforce", Veeco Instruments, Santa Barbara, CA, USA. All experiments were performed using V-shaped Si_3N_4 (silicon nitride) cantilevers (Veeco) having a length of 200 μm and a nominal spring constant of 0.06 N/m. The spring constant of each cantilever was calibrated just prior to the measurements using the built-in calibration procedure of Nanoscope IV Picoforce AFM.

Covalent attachment of proteins onto the tip and sample surfaces without special linkers was used. (Currently we are repeating this and some other force spectroscopy experiments using benzaldehyde-PEG6-NHS linkers (Ebner *et al.*, 2008), these results will be published elsewhere). Very briefly, the procedure of tip/sample functionalization was as follows. One percentage solution of glutaraldehyde (Sigma) was used as a coupling agent when functionalizing tips with barnase with the concentration of 250 mg/L after their initial intensive cleansing. The loosely attached proteins were then removed by extensive washing with PBS buffer. The protein-functionalized tips were used immediately for making measurements. Substrates (freshly cleaved muscovite mica) were functionalized for 5 min, followed by processing in a 1% v/v 3-aminopropyltriethoxysilane (APTES, Sigma) solution in water. This procedure was followed by processing the substrate in a 1% v/v glutaraldehyde solution in water for 15 min. After rinsing with deionized ultra high quality (UHQ) (resistivity 18 MΩ·cm) water, the samples were immersed into a solution of barstar with the concentration of 250 mg/L for 15 min. The non-reacted and loosely bonded proteins were subsequently removed by extensive washing with PBS buffer.

AFM images of mica surface functionalized in the aforementioned way demonstrate the formation of smooth protein monolayers (single molecules are seen as globules) with an average thickness of a few nanometers; for reference see e.g., Figure 2 from the paper (Chtcheglova *et al.*, 2004). Our previous single molecule force spectroscopy studies as well as literary data attest that for the surface functionalization method and protein concentrations used, mostly single- and double-molecule interactions are observed. In particular, and this was checked up again in these series of experiments, the lowering of concentration does not lead to the appearance of peaks with lower specific force in force histograms but strongly decreases the percentage of the successful approach—contract cycles, that is such cycles where specific interaction events are recorded. For the concentrations used, the percentage of successful cycles is

View this article online at wileyonlinelibrary.com Copyright © 2010 John Wiley & Sons, Ltd.

Journal of Molecular Recognition

20–25% for the 1000–1500 total number of cycles performed with the same cantilever/sample pair.

Typical pulling off (contraction) curve characteristic for the single molecule barnase–barstar interaction ("specific interaction event") is presented in Figure 1. Fuzzy logic-based software by Kasas *et al.*, 2000, was used to process such a data semiautomatically. This software assigns a grade ranging from 0 to 1 to each measurement, which could be interpreted as a "specific interaction event". The main criteria to give such a grade are the following. One criterion is the "quasiverticality" of the signal jump associated with the bond rupture (which takes place at $Z = 120$ nm in Figure 1). Relaxation of the cantilever position after such a rupture is a very fast process, which, for the determined by the given force loading rate scale of Figure 1 and similar graphs, necessitates such a quasiverticality. After the jump, force curve is to be seen as a horizontal straight line and thus should constitute approximately a right angle with the quasivertical "jumping part" of the curve. The position of this same straight line determines the zero force level, and the value of the signal change at the moment of the rupture is taken as a value of the specific interaction force. Further, the part of the force curve just before the rupture (which corresponds to Z position between 110 and 120 nm in Figure 1) should be seen as an approximately straight line without peculiarities. For each measurement, the software determines an average slope of this part of the curve (initially in Newton par nanometer), which then can be easily recalculated into Newton per second using the known value of the pre-established Z-scanning rate (in nm/s) amply determined by the full amplitude of the Z-displacement of the piezo and the force ramp repetition rate. This same slope is then taken as a physically sound force loading rate, which should not be mixed up with still often used nominal loading rate formally determined from the Z-scanning rate and cantilever spring constant.

An example of the force histogram is presented in Figure 2. To be sure that we measured specific interactions, different control experiments have been performed at the same conditions. As usual, first of all this includes the study of the "partial system", i.e., the study of an interaction between non-functionalized mica and tip, functionalized mica and non-functionalized tip, non-functionalized

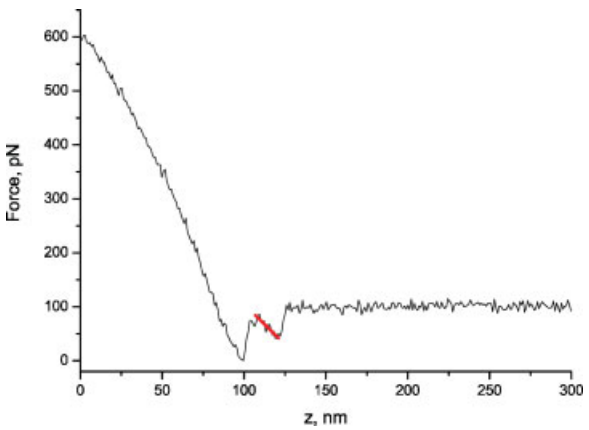

Figure 1. Typical force curve of the specific interaction between barnase and barstar. The first minimum of the force curve (around $Z = 100$ nm) corresponds to the non-specific adhesion and hence it is disregarded by our data processing software (Kasas *et al.*, 2000); the second minimum ($Z = 120$ nm) is a specific interaction. The approximation of the "close to the bond rupture" part of the force curve by a straight line whose slope is used to determine an actual value of the force loading rate is shown.

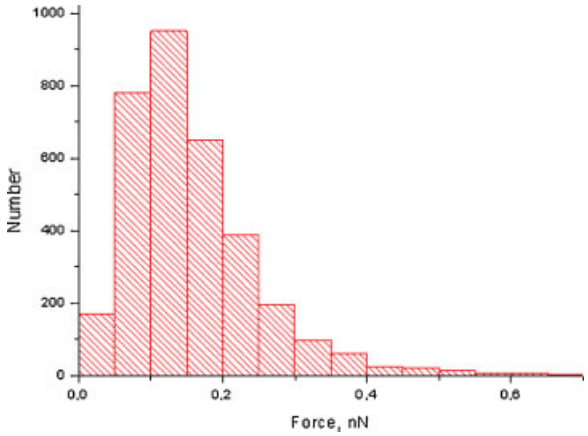

Figure 2. Force histogram corresponding to the force loading rate of $14 - 16$ nN/s.

mica and functionalized tip. Second, we have searched for specific interactions in the protein pairs barnase–barnase and barnase–avidin. Not less than 500 force curves were analyzed for any case. All of those yielded negative results: only rare (overall less than for the 1% of all approach-contraction cycles) events mimicking the specific interaction was observed for any control experiment. This is an unavoidable noise, and such a situation is quite common for the field. Similar decrease of the number of observations of specific interaction events was observed when free barnase or barstar with the concentration of 250 mg/L was added to solution to block the possibility of receptor-ligand observation during the approach-contraction cycles.

We do not see the necessity to present all this huge and uninformative data massive in the paper, hence for an illustration, in Figure 3 we reproduce only the results of the testing of the following partial system (more than one thousand of approach-contraction curves were recorded): AFM tips functionalized with barstar versus samples without any protein deposited but treated with APTES exactly in the same fashion as this is done for other experiments.

EXPERIMENTAL RESULTS AND DISCUSSION

The main results of our study are presented in Figures 2–4. In Figure 4 we present the dependence of the specific interaction force F^* on the force-loading rate $\dot{F}$ (in N/s) obtained for experiments performed in a PBS buffer solution (50 mM phosphate, 150 mM NaCl, pH 7.4 at 25°C). Each point on this graph is an average of no less than 250 specific interaction events. As an example, in Figure 2 we present a force histogram corresponding to the force loading rate ranging $14 - 16$ nN/s.

Experimental data is well approximated by a single straight line $F^* = a \ln \dot{F} + b$ with $a = 33$ pN, $b = 710$ pN. According to the Bell theory of the dependence of the specific interaction force (i.e., the most probable single bond rupture force) on the force-loading rate, such a dependence is given by the relation (Bell, 1978; Grubmueller *et al.*, 1996; Evans and Ritchie, 1997; Schwesinger *et al.*, 2000; Zlatanova *et al.*, 2000; Friedsam *et al.*, 2003; Weisel *et al.*, 2003; Lee *et al.*, 2007)

$$F^* = \frac{k_B T}{\Delta x} \ln \dot{F} + \frac{k_B T}{\Delta x} \ln \left(\frac{\Delta x}{k^*_{off} k_B T} \right) \quad (1)$$

Here k_B is a Boltzmann constant, T is a temperature, Δx is a barrier thickness and k^*_{off} is the natural off-rate, i.e., the dissociation rate

 Copyright © 2010 John Wiley & Sons, Ltd. View this article online at wileyonlinelibrary.com

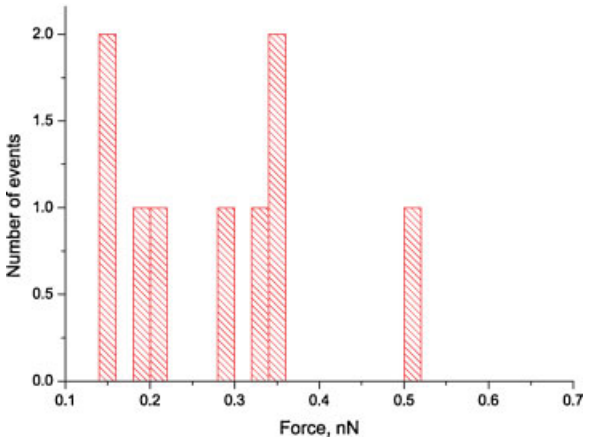

Figure 3. Force histogram of the control experiment performed with the following partial system: AFM tips functionalized with barnase versus samples without any protein deposited but treated with APTES exactly in the same fashion as this is done for other experiments. Force loading rate ranges $10-50\,\mathrm{nN/s}$.

where no external force is applied on the single molecule complex.

When applying this relation, one should have in mind that, first, it is derived for the condition that the loading rate $\dot{F}$ is independent on the force F, which is not exactly true (the part of the force curve just before the rupture, as for example the part corresponding to Z position between 40 and 70 nm in Figure 1, is only an *approximately* straight line). This relation also should be modified if more than one potential well (barrier) contribute to the force-induced complex dissociation (see e.g., (Dettmann *et al.*, 2000; Strunz *et al.*, 2000; Derenyi *et al.*, 2004) for some generalizations in this direction).

The values $\Delta x = 0.12\,\mathrm{nm}$ and $k^*_{\mathrm{off}} = 14\,\mathrm{s}^{-1}$ follow from the approximation of the data given before. To analyze the meaning

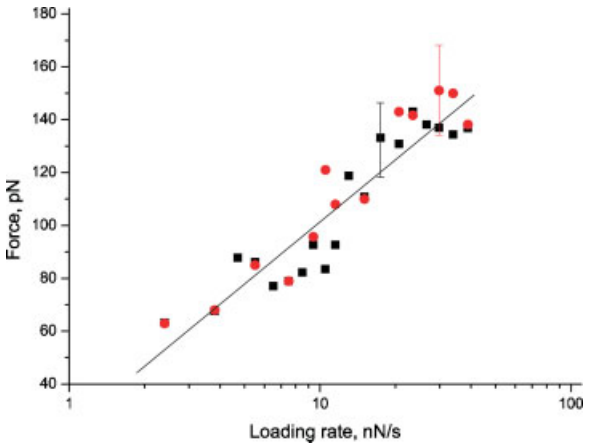

Figure 4. Dependence of the specific interaction between barnase and barstar on the force loading range. Black quadrants: experiments performed in PBS buffer solution (50 mM phosphate, 150 mM NaCl, pH 7.4 at 25°C), red circles: experiments performed in PBS buffer solution with additional NaCl (50 mM phosphate, 650 mM NaCl). Straight line shows the linear fit to the 150 mM NaCl data. Typical experimental errors of the specific force for both experiments are shown. The indicated value of the loading rate is just the central value of the interval of the loading rates used for the averaging which in typical case is equal to 2 nN/s.

of this data, first it looks instructive to compare them with those obtained for other receptor–ligand pairs. Although the most single molecule force spectroscopy experiments reveal the value of the barrier thickness Δx ranging 0.2–1 nm, *cf.* (Zlatanova *et al.*, 2000; Schwesinger *et al.*, 2000; Weisel *et al.*, 2003; Lee *et al.*, 2007), an observation of such narrow barriers as 0.05–0.15 nm is not uncommon in the field. In particular, they are characteristic for the most efficiently interacting streptavidin–biotin and avidin–biotin pairs, as this was reported, for different loading rates including the loading rate $\equiv 0$ (single complexes were kept stretched by a constant force (Favre *et al.*, 2007)), in (Yuan *et al.*, 2000; Lo *et al.*, 2001; Favre *et al.*, 2007). For example, as narrow barrier value as 0.024 nm was measured for avidin–biotin interaction for the force loading rate ranging 1.7–9.6 nN/s in de Odrowaz Piramowicz *et al.*, 2006. Similar barrier thicknesses were reported for some other ligand–receptor pairs as well, see e.g., Table 2 in the review Lee *et al.*, 2007.

At the first glance, the measured off rate, $k^*_{\mathrm{off}} = 14\,\mathrm{s}^{-1}$, appears to be too rapid for such efficiently interacting system as barnase–barstar: remind, that biochemical data attests for the same temperature and similar pH and ionic strength the off rate as slow as $8 \times 10^{-6}\,\mathrm{s}^{-1}$ (Schreiber and Fersht, 1993). By applying the Kramers theory of chemical bond dissociation in liquids $k^*_{\mathrm{off}} = \nu_0 \exp\left(-\frac{\Delta U}{k_B T}\right)$ with the typical pre-exponential factor $\nu_0 \cong 10^9 - 10^{10}\,\mathrm{s}^{-1}$ (Bell, 1978; Grubmueller *et al.*, 1996; Evans and Ritchie, 1997; Izrailev *et al.*, 1997), the latter value corresponds to the value of the dissociation barrier ΔU equal to $1.34 - 1.53 \times 10^{-19}\,\mathrm{J}$. In the same approximation our data gives only $0.75 - 0.85 \times 10^{-19}\,\mathrm{J}$ as the barrier value. Similar discrepancies with the biochemical data are not surprising and actually they are quite common for the single molecule force spectroscopy method: numerous examples can be found in reviews (Zlatanova *et al.*, 2000; Schwesinger *et al.*, 2000; Weisel *et al.*, 2003; Lee *et al.*, 2007) and other papers. Note, for instance, that comparably large off rates, ranging $1–30\,\mathrm{s}^{-1}$, have been earlier measured (again, for different force loading rates) by the force spectroscopy method even for the streptavidin/avidin–biotin pairs (de Odrowaz Piramowicz *et al.*, 2006). The reason of this discrepancy is, of course, the aforementioned insufficiency of the oversimplified single well (single barrier) model of the interprotein interactions which constitutes the basis for the derivation of Equation (1). This situation is illustrated by the model two-barrier energy landscape of Figure 5: if the whole landscape is composed by narrower internal and wider external wells, as it seems is often the case, already for rather small stretching forces the whole barrier ceases to contribute to the measured values, only the narrower internal barrier persists thus providing the experimental force spectroscopy data. Indeed, really slow force loading rates of the order of a few (tens) picoNewton per second are required to measure the whole "wide" barrier by the single molecule force spectroscopy method, *cf.* (Dettmann *et al.*, 2000; Strunz *et al.*, 2000; Derenyi *et al.*, 2004).

In the Introduction we have already discuss the inconsistency of the data pertaining to the contribution of the electrostatic interaction to the energy landscape of the barnase–barstar pair. This inconsistency makes difficult the detailed comparison of our experimental data with the theory, and for the current analysis we are obliged to limit ourselves with the conclusions that the total contribution of an electrostatic interaction is favorable and equal to a few kcal/mol (experimental data), and that the corresponding characteristic barrier width is a few nanometers large (for the latter the value of 3 nm has been derived in (Spaar *et al.*, 2006),

View this article online at wileyonlinelibrary.com Copyright © 2010 John Wiley & Sons, Ltd.

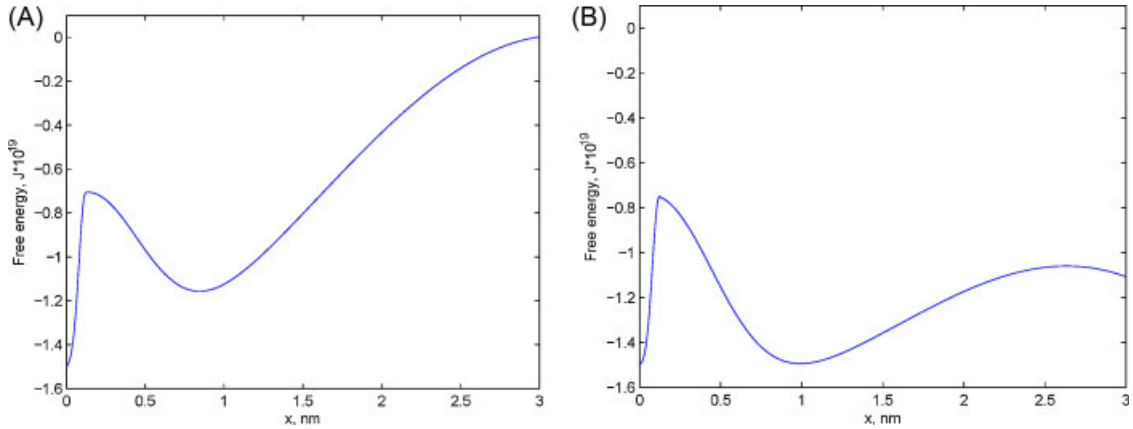

Figure 5. A graph illustrating the plausible energy landscape for the barnase–barstar interaction in absence (A) and presence of the applied force $F = 35$ pN (B). Experimental values for the internal barrier width and height taken from the present study, total barrier height from Schreiber and Fersht (1993) and electrostatic barrier width of 3 nm from Spaar *et al*. (2006) are used when preparing this graph. It is seen that already under the action of such a rather small force, the broad electrostatic interaction-related external well is not felt by a force spectroscopy method while the narrow internal barrier is only slightly modified.

see especially Figure 6 and Table 1 there). This means that as small stretching force as $\sim$ 30 pN already smears out an electrostatic interaction-related broad external barrier (*cf.* Figure 5). Not surprisingly that the latter cannot be seen in the force spectroscopy experiments performed with the force loading range lying in the nN/s range.

To check up this conclusion we performed numerous force spectroscopy experiments in such a manner that all experimental conditions apart from the salt concentration are the same. The salt concentration changes from zero (deionized ultra high quality (resistivity 18 MΩ·cm) water) to 700 mM (necessary quantity of salt was added to our PBS buffer solution). These experiments gave negative results: no statistically sound dependence of the force data on the salt concentration was observed. For example, for the 700 mM salt concentration the processing of the experimental data result in the values which are only *ca*. 10% different (this is less than an experimental error which is estimated as approximately 20%) from those reported above for the 150 mM salt concentration: $a = 29$ pN and $b = 700$ pN.

CONCLUSION

Single force spectroscopy data presented in this paper attest an efficient and highly specific interaction of the ribonuclease barnase with its inhibitor barstar and reveals an existence of narrow (with an effective width of 0.12 nm) and sufficiently high ($0.75 - 0.85 \times 10^{-19}$ J) "internal" well/barrier in their interaction energy landscape. Together with the "external" potential well given by the relatively long range electrostatic interaction, which can be estimated as $\sim 0.7 \times 10^{-19}$ J, these observations quite reasonably explain the overall dissociation barrier of the order of $1.34 - 1.53 \times 10^{-19}$ J inferred from experimental biochemical data.

No dependence of the specific interaction on the salt concentration was observed in our experiments. This is consistent with the relatively long range nature of the electrostatic interaction (it is only the latter which is influenced by the salt concentration via the changes of the charge screening range): such an interaction simply does not affect the data obtained in the force spectroscopy experiment performed at the typical conditions. Hence our results clearly demonstrate the possibility of the use of the force spectroscopy study to separate the short range "contact" and long range electrostatic interprotein interactions in a systematic way.

Acknowledgements

The authors thank G. A. Nalgranyan for the help during the beginning of the experiments. The financial support of Swiss National Science Foundation (grant no. 200020-119993), Russian Foundation for Basic Research (no. 09-04-01201-a) and Russian Federal Agency for Science and Innovation are gratefully acknowledged.

REFERENCES

Ababobu A, van der Vaart A, Gogonea V, Merz KM Jr. 2007. Interaction energy decomposition of protein-protein association: a quantum mechanical study of barnase-barstar complex. *Biophys. Chem.* **125**: 221–236.

Bell GI. 1978. Models of specific adhesion of cells to cells. *Science* **200**: 618–627.

Chong LT, Dempster SE, Hendsh ZS, Lee LP, Tidor B. 1998. Computation of electrostatic complements to proteins: a case of charge stabilized binding. *Protein Sci* **7**: 206–210.

Chtcheglova LA, Shubeita GT, Sekatskii SK, Dietler G. 2004. Force spectroscopy with a small dithering of AFM tip: a method of direct and continuous measurement of the spring constant of single molecules and molecular complexes. *Biophys. J.* **86**: 1177–1184.

de Odrowaz Piramowicz M, Czuba P, Targosz M, Burda K, Szymonski M. 2006. Dynamic force measurements of avidin – biotin and streptavidin – biotin interactions using AFM. *Acta Biochim. Polonica.* **53**: 93–100.

Derenyi I, Bartolo D, Ajdari A. 2004. Effectrs of intermediate bound states in dynamical force spectroscopy. *Biophys. J.* **86**: 1263–1269.

 Copyright © 2010 John Wiley & Sons, Ltd. View this article online at wileyonlinelibrary.com

Dettmann W, Crandbois M, André S, Benoit M, Wehle AK, Kaltner H, Gabius H-J, Gaub HE. 2000. Difference in zero-force and force-driven kinetics of ligand dissociation from beta-galactoside-specific proteins (plant and animal lectins, immunoglobulin G) monitored by plasmon resonance and dynamic single molecule force microscopy. *Arch. Biochem. Biophys.* **383**: 157–170.

Deyev SM, Waibel R, Lebedenko EN, Schubiger AP, Pluckthun A. 2003. Design of multivalent complexesusing the barnase-barstar module. *Nat. Biotechnol.* **21**: 1486–1492.

Dong F, Vijayakumar M, Zhou H-X. 2003. Comparison of calculation and experiment implicates significant electrostatic contributions to the binding stability of barnase and barstar. *Biophys. J.* **85**: 49–60.

Ebner A, Wildling L, Zhu R, Rankl C, Haselgrubler T, Hinterdorfer P, Gruber HJ. 2008. Functionalization of probe tips and supports for single-molecule recognition force microscopy. *Topics Cur. Chem.* **285**: 29–76.

Evans E, Ritchie K. 1997. Dynamic strength of molecular adhesion bonds. *Biophys. J.* **72**: 1541–1555.

Favre M, Chtcheglova LA, Lapshin DA, Sekatskii SK, Valle F, Dietler G. 2007. Force clamp spectroscopy with a small dithering of AFM tip and its application to explore an energy landscape of single avidin – biotin complex. *Ultramicroscopy* **107**: 882–886.

Fersht AA. 1993. Protein folding and stability - the pathway of folding of barnase. *FEBS Lett.* **325**: 5–16.

Florin E-L, Moy VT, Gaub HE. 1994. Adhesion bonds between individual ligand-receptor pairs. *Science* **264**: 415–417.

Friedsam C, Wehle AK, Kühner F, Gaub HE. 2003. Dynamic single-molecule force spectroscopy: bond rupture analysis with variable spacer length. *J. Phys.: Cond. Matt.* **15**: S1709–S1723.

Green NM. 1990. Avidin and streptavidin. *Meth. Enzymol.* **184**: 51–67.

Grubmueller HB, Heymann B, Tavan P. 1996. Ligand binding: molecular mechanics calculation of the streptavidin – biotin rupture force. *Science* **271**: 997–999.

Jucovic M, Hartley RW. 1996. Protein-protein interaction: a genetic selection for compensating mutations at the barnase-barstar interface. *Proc. Natl. Acad. Sci. USA* **93**: 2343–2347.

Hartley RW. 2001. Barnase-barstar interaction. *Meth. Enzymol.* **341**: 599–611.

Izrailev S, Stepaniants S, Balsera M, Oono Y, Schulten K. 1997. Molecular dynamics study of unbinding of the avidin – biotin complex. *Biophys. J.* **72**: 1568–1581.

Kasas S, Riederer BM, Catsicas S, Cappella B, Dietler G. 2000. Fuzzy logic algorithm to extract specific interaction forces from atomic force microscopy data. *Rev. Sci. Instrum.* **71**: 2082–2086.

Lee GU, Kidwell DA, Colton RJ. 1994. Sensing discrete streptavidin – biotin interactions with atomic force microscopy. *Langmuir* **10**: 354–357.

Lee LP, Tidor B. 2001a. Barstar is electrostatically optimized for tight binding to barstare. *Nat. Struct. Biol.* **8**: 73–76.

Lee LP, Tidor B. 2001b. Optimization of binding electrostatics: charge complementarity in the barnase-barstar protein complex. *Protein Sci.* **10**: 362–377.

Lee CK, Wang YM, Huang LS, Lin S. 2007. Atomic force microscopy: determination of unbinding force, off rate and energy barrier for protein – ligand interaction. *Micron* **38**: 446–461.

Lo Y-S, Zhu Y-J, Beebe TP Jr. 2001. Loading rate dependence of individual ligand – receptor bond – rupture forces studied by atomic force microscopy. *Langmuir* **17**: 3741–3748.

Mariani C, Gossele V, De Beuckeleer M, De Block M, Goldberg RB, De Greef W, Leemans J. 1992. A chimaeric ribonuclease inhibitor restors fertility to male steril plants. *Nature* **357**: 384–387.

Schneierman FB, Honig B. 2002. On the role of electrostatic interactions in the design of protein-protein interfaces. *J. Mol. Biol.* **318**: 161–177.

Schreiber G, Fersht AR. 1993. Inrteraction of barnase with its polypeptide inhibitor barstar studied by protein engineering. *Biochemistry* **32**: 5145–5150.

Schreiber G, Fersht AR. 1995. Energetics of protein-protein interactions: analysis of the barnase-barstar interface by single mutations and double mutant cycles. *J. Mol. Biol.* **248**: 478–486.

Schreiber G, Fersht AR. 1996. Rapid, electrostatically assisted association of proteins. *Nat. Struct. Biol.* **3**: 427–431.

Schulga A, Kurbanov F, Kirpichnikov M, Protasevich I, Lobachov V, Ranjbar B, Chekhov V, Polyakov K, Engelborghs Y, Makarov A. 1998. Comparative study of binase and barnase: experience in chimeric ribonucleases. *Protein Eng.* **11**: 775–782.

Schwesinger F, Ros R, Strunz T, Anselmetti D, Güntherodt H-J, Honegger A, Jermutus L, Tiefenauer L, Plücktun A. 2000. Unbinding forces of single antibody-antigen complexes correlate with their thermal dissociation rates. *Proc. Natl. Acad. Sci. USA* **97**: 9972–9977.

Spaar A, Dammer C, Gabdoulline RR, Wade RC, Helms V. 2006. Diffusional encounter of barnase and barstar. *Biophys J.* **90**: 1913–1924.

Strunz T, Orosan K, Shumakovich I, Guentherodt H-J, Hegner M. 2000. Model energy landscape and the force induced dissociation of ligand-receptor bonds. *Biophys. J.* **79**: 1206–1212.

Tang C, Iwahara J, Clore M. 2006. Visualization of transient encounter complexes in protein-protein association. *Nature* **444**: 383–386.

Wang T, Tomic S, Gabdoulline RR, Wade RC. 2004. How optimal are the binding energetics of barnase and barstar? *Biophys. J.* **87**: 1618–1630.

Weisel JW, Shuman H, Litvinov RI. 2003. Protein-protein unbinding induced by force: single molecule studies. *Curr. Opin. Struct. Biol.* **13**: 227–235.

Yuan C, Chen A, Kolb P, Moy VT. 2000. Energy landscape of streptavidine – biotin complexes measured by atomic force microscopy. *Biochemistry* **39**: 10219–10223.

Zlatanova JS, Lindsay M, Leuba SH. 2000. Single molecule force spectroscopy in biology using the atomic force microscope. *Prog. Biophys. Mol. Biol.* **74**: 37–61.

View this article online at wileyonlinelibrary.com Copyright © 2010 John Wiley & Sons, Ltd.